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Original Article

Staphylococcus aureus nasal colonization and strain concordance in patients with community associated Staphylococcal primary pyoderma - A cross-sectional study

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Abstract

Background Community associated Methicillin resistant Staphylococcus aureus (MRSA) are common among Staphylococcal isolates from skin and soft tissue infections (SSTI). We intended to find the current status of SA primary skin infection and the antibiotic resistance patterns of SA isolates in India. There is a paucity of data on the significance of nasal SA carriage in the pathogenesis of skin infections. Hence, we investigated the presence and association between nasal SA carriage and staphylococcal primary pyoderma.

Methods Hundred consecutive patients of primary pyoderma of all age groups and both sexes were recruited. Nasal swabs and pus samples from pyoderma lesions were taken for Gram's stain and bacterial culture. Antibiotic sensitivity patterns of nasal and pyoderma isolates were compared in patients with Staphylococcal primary pyoderma.

Results Furuncle was the most common presentation seen in 56%, followed by folliculitis (17%) and impetigo (15%). Of the various organisms isolated from pus and nose, SA (58% and 50% respectively) and Coagulase negative staphylococci (CONS) (22% and 36% respectively) were the most predominant. MRSA infection and MRSA nasal carriage were found in 35% and 25% of cases respectively. Nasal carriage of SA and MRSA were found to be significant risk factors for the development of SA (p-0.015) and MRSA pyodermas ($p<0.0001$) respectively. Phenotypic concordance of nasal and pus isolates were seen in 34.5% (20/58) of SA-pyodermas, 70% of which were MR.

Conclusion A high incidence of MRSA with resistance to commonly used antibiotics and high nasal SA carriage rates were observed by us, which is disturbing. Avoidance of inappropriate antibiotic usage is the need of the hour. Screening for nasal SA carriage may prevent recurrences and spread of CA-MRSA strains.

Key words
Primary pyoderma, staphylococcus aureus, MRSA, nasal carriage.

Introduction

Staphylococcus aureus (SA) is the commonest cause of pyodermas. Community associated Methicillin resistant *Staphylococcus aureus* (CA-MRSA) are common among SA isolates from skin and soft tissue infections (SSTI). although they rarely cause life-threatening infections.[1] Recent reports suggest that the introduction of CA-MRSA strains into hospitals is followed by severe infections in hospitalised patients.[1] Although CA-MRSA generally remain susceptible to several non-betalactam antibiotics when compared to hospital acquired methicillin

resistant Staphylococcus *aureus* (HA-MRSA), recent studies have shown increasing resistance.[1,2]

The ecological niche of SA are the anterior nares. Nose is regarded as the major site of carriage. Twenty percent of individuals are continuously colonized with *SA*, and occasional carriage is found in 60% of healthy people.[3] Nasal carriage has been found to be an important risk factor for infection in patients undergoing surgery, those on hemodialysis, those with intravascular devices, patients with HIV infection and those colonized with MRSA.[3] However, little is known about the significance of nasal carriage of SA in the pathogenesis of SA-SSTI. The incidence of subsequent infections is up to 4 times higher among MRSA carriers than methicillin-sensitive *Staphylococcus aureus* (MSSA) carriers.[4] Though there are many studies on nasal flora in normal and pyoderma patients,[3,4] there are very few comparing both isolates in the same patient.[5]

Aims and Objectives

We sought to study the antibiotic sensitivity patterns of SA strains isolated from patients with primary pyoderma and find the current status of methicillin resistance in them. We also investigated nasal SA carriage (both MRSA and MSSA) in patients with staphylococcal primary pyoderma and compared the antibiotic sensitivity patterns of nasal and pyoderma isolates. Any predisposing factors for MRSA infection or nasal colonisation like socioeconomic status, diabetes and nutritional status were also looked for.

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Materials and Methods

Hundred patients of all age groups and both genders attending the dermatology out-patient department of our hospital, diagnosed to have primary pyoderma between June 2015 to June 2016 were included in the study after written informed consent. The study was approved by Institutional Ethical Committee. Patients on immunosuppressives, those with a history of topical and systemic antibiotic use in the past 1 week and HIV positive patients were excluded.

Nasal swabs and pus samples from pyoderma lesions were taken for Gram's stain and bacterial culture. The samples were inoculated separately on 5% sheep blood agar and MacConkey's agar. Plates were incubated aerobically at 37°C for 18-24 hours and observed for growth. The bacterial strains were identified based on colony morphology and biochemical tests. Antibiotic susceptibility testing was performed by Kirby–Bauer disk diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines using a bacterial inoculum with turbidity equivalent to a McFarland turbidity standard of 0.5.[6] The bacterial isolates were tested for chloramphenicol, gentamycin, cefoxitin, clindamycin, erythromycin, vancomycin, tetracycline, linezolid and cotrimoxazole. Screening for methicillin resistance was done by cefoxitin (30 μg) sensitivity.

Nutritional assessment of all subjects was done using Body mass index (kg/m^2) as per WHO guidelines. For adults, a BMI of 18.5 to 24.99 was considered normal, BMI>=25 as overweight and >=30 as obese. For children and adolescents (till 19 years), BMI was calculated and overweight was taken as more than one standard deviation of body mass index for age and sex,

and obese as more than two standard deviations of body mass index for age and sex.[7] Socio-economic status assessment was done using modified B.G. Prasad classification.[8] All statistical tests were carried out using software SPSS for windows (version 21.0), and Chi square test was used for comparison of the variables, p value less than 0.05 was considered statistically significant.

Results

After obtaining informed consent from patients and parents (in case of children), 100 cases of primary pyoderma, fulfilling inclusion criteria, were included in the study. The age-group ranged from 1 to 80 years with a mean age of 28.50±19.103 yrs. Patients below 20 years (45%) and males (64%) formed the majority of cases **(Table 1)**. Furuncle was the most common presentation seen in 56%, followed by folliculitis (17%) and impetigo (15%) **(Figure 1)**. Hair follicle infection constituted majority of primary pyodermas (73%) **(Figure 2)** and the most common site affected was lower limbs in 48%, followed by head and neck (31%). 69% cases were normal in weight, 21% over weight and 10% under-weight. Economically, majority were in the lower income group (63%) and 8% were diabetics **(Table 1)**.

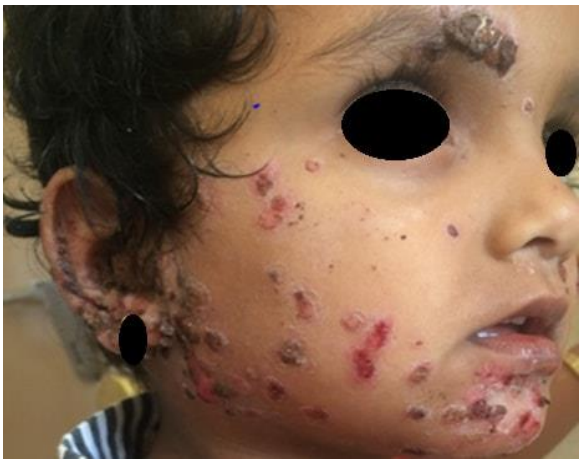

Figure1 Impetigo on the face in a child.

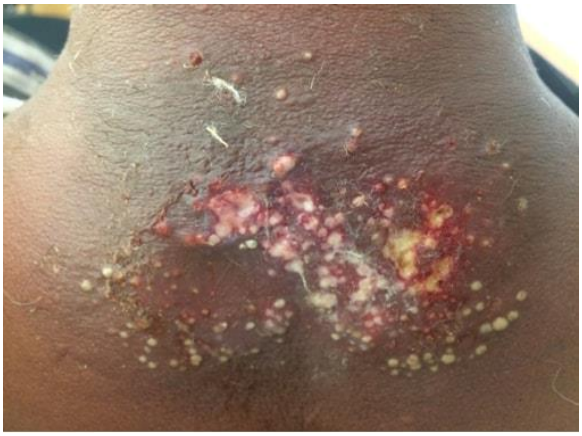

Figure 2 Carbuncle over the back in a diabetic male.

Table 1 Demographic data and bacterial culture findings of the study population.

Parameter		*No.*
Age	1-20	45
	20-40	27
	40-60	23
	>60	5
Sex	Female	36
	Male	64
Diagnosis	Faruncle	56
	Folliculitis	17
	Impetigo	15
	Carbuncle	4
	Paronychia	5
	Erysipelas	2
	Abscess	1
BMI	Normal	68
	Overweight	20
	Underweight	11
Socioeconomic status	Upper middle	37
	Lower middle	30
	Poor	33
DM	Yes	9
	No	91
Site of involvement	Head and Neck	31
	Lower limb	48
	Upper limb	11
	Trunk	10
Pus culture	Staphylococcus aureus	58
	Coagulase negative staphylococci	22
	Beta-hemolytic streptococci	7
	Klebsiella	2
	Non-hemolytic streptococci	3
	Citrobacter	0
	No growth	8
Nasal culture	Staphylococcus aureus	50
	Coagulase negative staphylococci	36
	Klebsiella	6
	E.coli	2
	Non-hemolytic streptococci	3
	Citrobacter	1
	No growth	2

Table 2 The antibiotic sensitivity pattern of *Staphylococcus aureus* isolated from pyoderma and nose of patients with skin and soft tissue infections.

Antibiotic tested	*Pus SA*		*Nose SA*	
	Sensitive n (%)	*Resistant n (%)*	*Sensitive n (%)*	*Resistant n (%)*
Cefoxitin	23(39.7%)	35(60.3%)	25(50%)	25(50%)
Linezolid	58(100%)	0(0%)	50(100%)	0(0%)
Vancomycin	36(62%)	22(38%)	35(70%)	15(30%)
Tetracycline	28(48.3%)	30(51.7%)	32(64%)	18(36%)
Cotrimoxazole	39(67.2%)	18(32.8%)	26(52%)	24(48%)
Clindamycin	37(63.8%)	21(36.2%)	31(62%)	19(38%)
Gentamycin	23(39.7%)	35(60.3%)	28(56%)	22(44%)
Erythromycin	18(31%)	40(69%)	13(26%)	37(74%)
Chloramphenicol	45(77.6%)	13(22.4%)	38(76%)	12(24%)

Table 3 Association of pus SA and MRSA with nasal colonization.

	SA pus (n=58)		*MRSA pus (n=35)*	
	Negative	*Positive*	*Negative*	*Positive*
SA nose(n=50)				
Negative	27(64.3%)	23(39.7%)	-	-
Positive	15(35.7%)	35(60.3%)	-	-
P value	0.015		-	-
MRSA	-	-		
nose(n=25)	-	-	23(54.8%)	19(45.2%)
Negative	-	-	0(0%)	16(100%)
Positive				
P value	-	-	<0.0001	

SA- Staphylococcus aureus, MRSA- Methicillin resistant Staphylococcus aureus.

We found Gram's stain positivity in pus samples of 86 cases, 14 were sterile. Amongst the positive smears, 28 showed cocci in pairs, 24 in clusters, 4 in singles, 30 samples showed more than one type of arrangement of gram-positive cocci and 1 smear had gram negative bacilli. Similarly, 88 nose samples were positive and 12 were sterile on Gram's stain. Of the positive nasal smears, 24 showed gram-positive cocci in pairs, 33 in clusters, 14 in single, 17 showed more than one type of arrangement of gram-positive cocci and 7 smears had gram negative bacilli, on culture, 92/100 pyoderma samples and 98/100 nose samples yielded growth. Of the various organisms isolated from pus and nose, SA (58% and 50% respectively) and Coagulase negative staphylococci (CONS) (22% and 36% respectively) were the most predominant **(Table 1)**. The sensitivity patterns of SA strains from pyoderma and nose are given in **Table 2**. Methicillin resistance was seen in 35 (60.3%) cases of SA pyoderma, whereas 25 (50%) nasal SA isolates were MR. All SA isolates were sensitive to linezolid. 35 (%) out of 58 (60.3%) SA pyoderma patients were nasal carriers of SA. Nasal carriage of SA was found to be a significant risk factor for the development of Staph. Pyoderma (p-0.015) **(Table 3)**. Among pus CONS isolates, all were sensitive to linezolid and 13(59%) were sensitive to tetracycline and cotrimoxazole. Methicillin resistance was seen in 16 (72.7%) CONS isolates.

The sensitivity of pus and nasal MRSA strains to various antibiotics are compared in **Figure 3**. All were sensitive to linezolid. Sensitivity to cotrimoxazole was more in pus MRSA (63%), while vancomycin sensitivity was more in nasal MRSA (72%). Sixteen out of 35 MRSA pyoderma cases were nasal carriers of MRSA. Nasal MRSA carriage was found to be a highly significant risk factor for development of MRSA pyoderma (p<0.0001) **(Table 3)**. When age, sex,

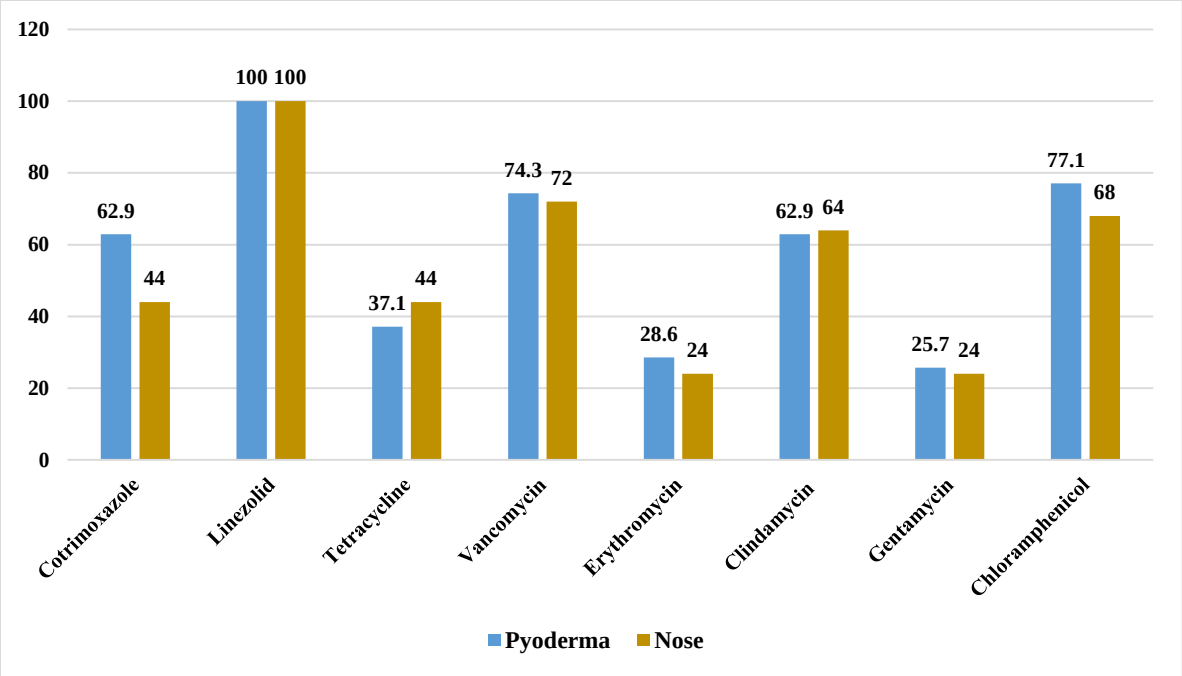

Figure 3 Compound bar diagram depicting the comparison of antibiotic sensitivity patterns of pus and nasal MRSA strains.

Table 4 Assessment of risk factors for MRSA pyoderma and MRSA nasal carriage.

Parameter		*MRSA from pyoderma*			*MRSA from nose*		
		No	*Yes*	*p value*	*No*	*Yes*	*p value*
Sex	Female	23(63.9)	13(36.1)	0.9	25	11	0.3
	Male	42(65.6)	22(34.4)		50	14	
BMI	Normal	45(66.2)	23(33.8)	0.8	53	15	0.5
	Overweight	12(60)	8(40)		13	7	
	Underweight	8(72.7	3(27.3)		8	3	
Socioeconomic	Upper Middle	25(67.6)	12(32.4)	0.8	26	11	
	Lower	18(60)	12(40)		20	10	0.1
	Poor	22(66.7)	11(33.3)		29	4	
Site	HN	23(74.2)	8(25.8)		22	9	
	LL	29(60.4)	19(39.6)	0.1	36	12	0.2
	TR	4(40)	6(60)		6	4	
	UL	9(81.8)	2(18.2)		11	0	
DM	No DM	60(65.9)	31(34.1)	0.5	67	24	0.3
	DM	5(55.6)	4(44.4)		8	1	
Diagnosis	Furuncle	28(50)	28(50)		42	14	
	Folliculitis	13(76.5)	4(23.5)		13	4	0.2
	Impetigo	12(80)	3(20)	0.025	12	3	
	Carbuncle	4(100))	0(0)		2	2	
	Paronychia	5(100)	0(0)		5	0	
	Erysipelas	2(100)	0(0)		0	2	
	Abscess	1(100)	0(0)		1	0	

BMI, site of involvement, type of pyoderma or the presence of diabetes were compared between MRSA and non- MRSA pyoderma cases, there was no statistically significant difference. Similarly, we did not find any significant difference in these parameters between MRSA and non- MRSA nasal carriers **(Table 4)**.

Pyoderma and nose samples grew the same organism in 42 cases (SA-34, CONS-8). Congruence of antibiotic sensitivity pattern of pus and nasal SA isolates was seen in 20% of primary pyodermas, 14 of which were MR. (70%). All the identical isolates were sensitive to vancomycin and linezolid (100%), 80% showed sensitivity to tetracycline, cotrimoxazole and clindamycin. There was no significant association of the identical isolates with age, sex, site of involvement, diagnosis, nutritional state, socio-economic status or the presence of diabetes mellitus (p value>0.05).

Discussion

There is an enhanced emergence of drug resistant strains in primary pyoderma, complicating treatment and control.[2,9] Primary pyodermas are common in patients less than 20 years, as observed by us and others.[5] This has been attributed to poorly developed epidermal barrier in children.[5] A male preponderance was observed by us, consistent with other studies.[2,10,11] This could be due to higher chance of occupational trauma and exposure to bacteria in males. There is evidence that males carry higher numbers of bacteria (aerobic) than females.[12]

Furuncle has been found to be the predominant clinical presentation, followed by folliculitis and impetigo in studies from India on primary pyodermas, similar to our observations.[13,14] Some have found folliculitis [10] and impetigo to be more common.[11,15] In contrast, abscesses were found to be the commonest type of presentation in a multicentre study from United States.[16] Pyodermas commonly involve lower limbs, head and neck.[16] Clustering of lesions to the extremities, as seen by us has been reported in previous studies on primary pyoderma.[2]

The Gram stain remains the first diagnostic test in the processing of pus specimens in the laboratory and gives physician preliminary information about the presence and cause of infection. In our study, 86% of pus samples were smear positive, whereas 92% yielded growth on bacterial culture. The lesser smear positivity could be due to technical errors, inadequate material and lack of expertise. The culture positivity rates in primary pyoderma have varied from 83.7% to 96% in several studies.[10,11,14] Primary pyodermas are often monomicrobial in etiology, rarely polymicrobial. Most of our isolates (99%) were monomicrobial consistent with that observed by others.[10,13-15] *Staphylococcus aureus* has been found to be the predominant pathogen in both primary and secondary community associated pyodermas. It has been isolated in around 80% of cases of pyodermas in previous studies.[10,14,15] We grew coagulase positive *S. aureus* in 58% cases, which is lower than prior reports. CONS isolation rates in pyodermas have ranged from 3%[15] to 16.39%,[17] we grew CONS in 22% cases. *Streptococci* are less commonly isolated from pyodermas, the rates vary from 2[10,13,14] to 9%,[16] we found them in 6% of cases. Gram negative organisms like *Pseudomonas, E. coli, enterococci* have been isolated rarely in a few studies.[14,15] *Klebsiella* was grown by us in 2% cases.

Antibiotic resistance patterns of Staphylococci from pyoderma and nose

In our study, 60.3% SA isolates from pyoderma were methicillin resistant and 38% were vancomycin resistant. A zero resistance to vancomycin has been reported in some studies.[10,15] Around 1/3 (23-26%) of our SA strains were resistant to cotrimoxazole and clindamycin.[15,18] Clindamycin resistance has been found to be 2.4% and 14.5% in an Indian and Chines study respectively.[15,19] We observed

tetracycline and macrolide resistance in two-thirds of the isolates (60-70%), similar to Nagaraju *et al*.[18] However, some have found sensitivity to tetracyclines and macrolides in more than two-third isolates.[2,10,15] Gentamicin resistance was also found to be higher in our study (60 %) compared to others.[10,15,18] All our pus SA isolates (both MSSA and MRSA) were linezolid sensitive similar to prior observations.[9,14-16] Among CONS isolates from pus, 33% were MR.

Fifty patients grew SA in the nose, of which 50% were methicillin resistant. Nose SA isolates were sensitive to all antibiotics tested in more than half of the cases, except erythromycin, to which only 26% strains were sensitive. We grew CONS from nose in 40 cases, 18 of which were MR. Acquisition of different Staphylococus cassette chromosome SCC *mec* elements(theses SCC mec elements carry the mecA gene which is responsible for methicillin resistance) by SA has led to the emergence of HA-MRSA and CA-MRSA strains; methicillin-resistant CONS are thought to represent a reservoir of SCC *mec* for CA-MRSA.[20]

MRSA from pyoderma and nose

Many reports from India and Asia have highlighted the prevalence of MRSA. According to National Staphylococcal Phage Typing Centre India, there is a consistent increase in the occurrence of MRSA from 9.83% in 1992 to 45.44% in 1998. MRSA strains were more common in southern India (30.94%) than in the west (20.33%) or north (18.88%).[21] In SSTI, the prevalence of CA-MRSA around the world has been variable from 2.6% (China),[22] 61%(USA)[16] to 74% (Taiwan).[23] Similarly, in India, prevalence varies from 9.6% (Delhi),[15] to 61% (Kashmir).[14] Surprisingly, Patil *et al*. found all but one strain to be MSSA while studying primary pyoderma cases from West India.[10] We found 60.3% isolates to be MRSA, similar to the findings of Bhat *et al*. Our study data suggest an increased prevalence of MRSA in primary pyodermas in our part of South India.

All pus and nose MRSA strains were sensitive to linezolid similar to the findings of Emilda *et al*. Surprisingly, we found vancomycin resistance in 25.7% of pus MRSA while other studies from India and US have observed zero resistance to vancomycin.[9,4,16] Sensitivity to cotrimoxazole has been found to vary from 57%[2,9] to 100%.[16] We also found a 63% sensitivity to cotrimoxazole.[17] Clindamycin sensitivity in pyodermas has been observed to range from 69.3% to 93% in various studies across the world,[14,16,19] we found 63% strains to be clindamycin sensitive. Hence, clindamycin and cotrimoxazole can be considered as good alternatives to linezolid in MRSA pyoderma. Around 2/3rds of pus MRSA were found to be resistant to tetracycline and gentamicin by us, while others have observed a12.5%[16] to 43.8%[19] tetracycline resistance rate. Erythromycin resistance was found in 71.4% of MRSA by us, while another Indian study observed 87.5% erythromycin resistance.[15] Overall, a higher resistance to all antibiotics tested was observed by us compared to previous studies. The generous use of antibiotics and lack of a proper antibiotic policy in our country could explain this.

Nasal carriage of SA

Nasal carriage of SA was found in 60.3% of SA pyoderma patients, similar to Nagaraju *et al*. (54.4%).[18] This is in slight variation to observations in studies from United States, wherein carriage rates of 70%[24] and 88.2%[25] were found. Among the hundred primary pyoderma cases, 25% were found to be CA-MRSA nasal carriers compared to 6-8% nasal

carriage rates found by others from India.[8] The nasal MRSA carriage rates in SA and MRSA pyoderma patients were 27.6% and 45.7% respectively. Another Indian study found a lesser carriage rate (13.5%) in MRSA pyodermas.[9] In the United States, MRSA nasal carriage rates amongst MRSA SSTI cases has been found to be variable from 25% to 67.3%.[25,26]

Risk factors for SA and MRSA infection

Lee *et al*.[27] found history of prior SSTIs and male gender as risk factors for the acquisition of SSTI. No such significant association was found in our study. The CDC have proposed five factors or five Cs of MRSA transmissions such as crowding, frequent skin-to-skin contact, compromised skin integrity, contaminated items and surfaces and lack of cleanliness.[28] Majority of our cases (63%) belonged to lower middle or poor socioeconomic status, where overcrowding and lack of cleanliness is prevalent. This could have contributed to high incidence of MRSA transmission in them, which however was not statistically significant. Male gender, nasal carriage of MRSA, exposure to an individual who had surgery or antibiotic treatment for SSTI within a year before infection were identified as significant risk factors for MRSA SSTI in a Taiwan study.[23] Male gender was not found to have a significant association with MRSA by us.

Nasal MRSA carriage was a highly significant risk factor for development of MRSA pyoderma ($p<0.0001$) in our study. A similar observation was made in another study.[29]

MRSA colonization was observed to be most likely in the groin, followed by nose and rectum, among those with MRSASSTI by Albrecht *et al*.[25] Some also suggest that non-nasal colonization may play a key role in CA-MRSA transmission and infection.[26] We did not check for non-nasal colonisation in our patients.

Strain concordance of SA isolates from pus and nose

The identity of strains from pus and nose can be demonstrated by determining the phenotypic properties or by genotyping of the isolates. Phenotypic properties include biochemical tests and, antibiotic resistance data. They are widely used, cheaper, detect only viable bacteria, and yield isolates that can further be characterised and studied.[30] Phenotypic concordance of nose and pus isolates was seen in 34.5% (20/58) of our SA-pyoderma patients, of which 70% were MR. This is in concurrence with the findings of Nagaraju *et al*.[18] Molecular techniques have the advantage that, they are rapid, less laborious, and more sensitive, specific and efficient compared to the conventional method.[30] Ellis *et al*.[24] found phenotypic concordance to be more common in MSSA SSTI and concordance by molecular typing to be more in MRSA SSTI. We did not do molecular analysis of SA strains. We observed greater phenotypic concordance in patients in MRSA 40% (14/35) than MSSA pyoderma 26.1% (6/23).

Limitation

Testing for non-nasal colonization and molecular typing of bacterial isolates from pyoderma and nose was not done by us.

Conclusion

Staphylococcus aureus still remains the major causative agent of primary pyodermas in the community. A high incidence of MRSA SSTI and high nasal MRSA carriage rates, along with an increased resistance to commonly used antibiotics which is disturbing. A proper antimicrobial policy and avoidance of inappropriate antibiotic usage is the need of the hour to reduce resistant CA-MRSA infections. Pus culture sensitivity and screening for nasal

SA carriage is essential in primary pyoderma cases. This may prevent recurrences and spread of CA-MRSA strains.

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